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Influence of Methyl *tert*-Butyl Ether on Lake Water Algae

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Methyl *tert*-butyl ether (MTBE) has been used as an octane booster in gasoline in the United States since the 1970s. MTBE use increased greatly in the 1990s with the implementation of the Clean Air Act Amendments of 1990. The MTBE enhanced a more complete combustion of fuel hydrocarbons with reduced carbon monoxide and ozone levels in urban air. By 1998, MTBE production was fourth most produced chemicals in the USA (Johnson et al. 2000). MTBE has been detected in surface and shallow well samples since 1993 (Newman 1995). Although MTBE is less toxic than some other gasoline components, it can cause a taste and odor problem in water supplies. Some animal exposure studies have indicated that MTBE is a carcinogen. MTBE is an aliphatic hydrocarbon ether with high solubility in water, and it does not biodegrade readily in a subsurface environments because of its chemical structure (Andrews 1998; Johnson et al. 2000). Sources of MTBE contamination in water bodies are releases from gasoline-powered watercraft, atmospheric deposition, precipitation of industrial vapors, and vehicle emissions (Reuter et al. 1988). Presently about 24 percent of all gasoline contain about 11 percent by volume MTBE in the USA (Herrick 2000).

This study was done to determine if MTBE contamination had an adverse influence on algae in Lake Texoma waters. Powerboat activities at some Lake Texoma marinas have caused a localized contamination of MTBE in the water. Water samples from powerboat active marinas were spiked with different concentrations of MTBE. Toxic levels of MTBE were determined by measuring the viability of algae by monitoring chlorophyll-*a*, which reverts to phaeophytin-*a* upon death of the algae.

MATERIALS AND METHODS

The lake water samples used for testing were collected from the top foot of water at three marinas called Soldier Creek (on June 12, 2000), Cedar Mills (on June 26, 2000) and Catfish Bay (on July 10, 2000). The toxicity tests were conducted in a closed batch system at the ambient laboratory temperature of 24 ± 1 °C.

Light source used was continuous blue plant light from a GE 65 Watt reflector

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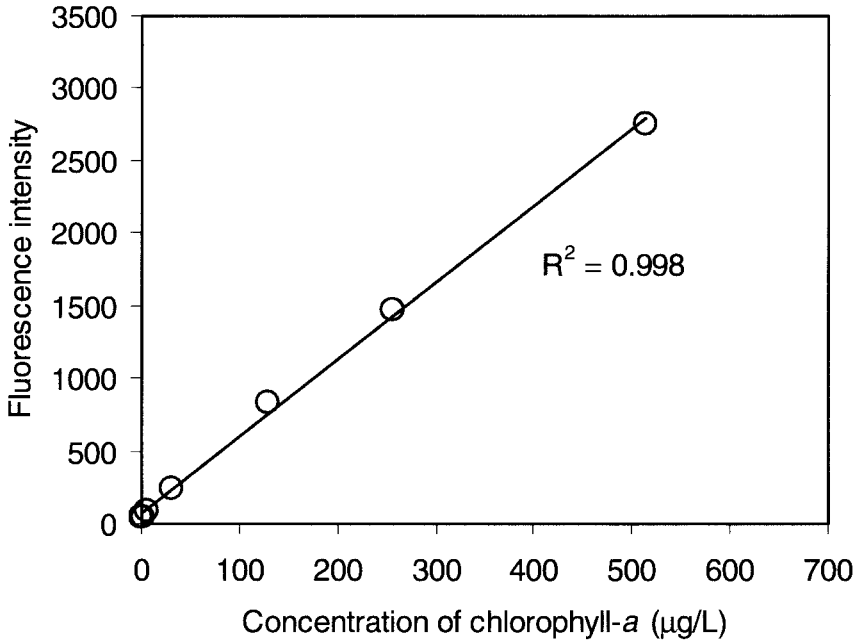

Figure 1. Fluorescence intensity as a function of chlorophyll-*a* concentrations at room temperature. Symbols are experimental data, and lines are fitted fluorescence intensities. Excitation and emission wavelength for chlorophyll-*a* were 440 and 665 nm, respectively.

lamp. Clear glass jars (polypropylene open-top cap with silicone septum, volume = 135 mL) were used as sample containers. The appropriate amount of MTBE was added to replicate set of jars. Controls without MTBE were prepared because chlorophyll-*a* is light sensitive so it would decompose by light with time. On initial day 0, sealed glass jars without headspace were labeled for MTBE concentration, days of incubation and sample number. Samples identified as day 0 were analyzed for chlorophyll-*a*. All other sample jars were incubated cap down to minimize the loss of MTBE by volatilization. They were placed in an air exhaust hood to avoid exposure from any hazardous compounds. Daily the samples were shaken by hand for a few seconds. Hood temperature was recorded daily. In Test 1, as a preliminary experiment with Soldier Creek marina waters, MTBE concentrations of 0, 10, and 3000 mg/L were prepared as duplicate samples. Chlorophyll-a concentrations were monitored up to 9 days. This test was done without exposure to the blue plant light. In Test 2 with Cedar Mills marina waters, MTBE concentrations prepared were 0, 10, 10^2, 10^3, and 10^4 mg/L in triplicate containers. In Test 3 with Catfish Bay marina waters, the MTBE concentrations used ranged from 2000 to 10000 mg/L and were prepared in duplicate. After an incubation period of one to ten days, the samples were analyzed for chlorophyll-*a*.

Purified chlorophyll-*a* was used from Sigma Chemical Co. (#25825-3) as a standard. All samples were vacuum filtered through glass fiber filters (Whatman, 47mm). Filters containing the chlorophyll-*a* residue were stored in a closed jar with silica gel desiccant and kept in a freezer before extraction. Chlorophyll *a* was then extracted from the filter residue using 90 % heated ethanol (HPLC grade). The extract was kept for 24 hours in the dark at room temperature (Sartory and Grobbelaar 1984). All extracts were centrifuged for 30 minutes at 1500 rpm using a Beckman GS-6KR Centrifuge. An aliquot of the supernatant was transferred to a round borosilicate cuvette (12 × 75 mm, 1 – 5 mL capacity) and fluorescence was measured using a Turner Quantech Digital Field fluorometer (FM 109525). Excitation and emission wavelength for chlorophyll *a* were 440 and 665 nm, respectively. Standards were also prepared in 90% ethanol (HPLC grade) and the concentrations were measured using UV spectrophotometer (Milton Roy Spectronic 21). The molar absorptivity of chlorophyll-*a* used for ethanol extracts was 3841 M^{-1} cm^{-1} at 665 nm (Wintermans and De Mots 1965). A calibration curve shown in Figure 1 was used to calculate chlorophyll-*a* concentration in the test samples using the extract volume (10 mL) and sample volume filtered (135 mL).

RESULTS AND DISCUSSION

Figure 2 shows average chlorophyll-*a* concentrations of Lake Texoma water collected from Soldier Creek marina as a function of MTBE concentrations. Initial chlorophyll-*a* concentration measured was 20.8 µg/L. There was not an observed difference of chlorophyll-*a* levels between 0 and 3000 mg/L MTBE. This indicated that MTBE used would not be toxic to the lake water algae unless MTBE water concentrations were greater than 3000 mg/L.

Figure 3 shows average chlorophyll-*a* concentrations of Lake Texoma water collected from Cedar Mills marina as a function of MTBE concentrations. Initial chlorophyll-*a* concentration measured was 26.6 µg/L. There was a similar gradual decline in the viable algae at spike amounts of 0, 10, 10^2, and 10^3 mg MTBE/L. After ten days incubation the lower levels of MTBE may have enhanced algae viability over the control. This was consistent with results obtained in Test 1. These phenomena may have occurred by restricting protozoa activity that grazes on algae. In addition, the presence of residual methanol or *tert*-butyl alcohol in MTBE or oxidation byproduct may serve as a carbon source for algal growth (Rousch and Sommerfeld 1998). The highest concentration of 10,000 mg/L did show a drastic adverse effect on algae viability.

Test 3 was conducted with some fresh lake water from Catish Bay marina to show the influence between MTBE concentrations of 2000 and 10,000 mg/L. Algae activity was markedly restricted as MTBE levels increased through the concentrations of 4000, 6000, up to 10,000 mg/L (Figure 4). Extrapolation of the plots in Figure 4 indicated that complete reduction of algae activity would occur in 3 ½ days at 10,000, 6 days at 8000, about 9 days at 6000, and about 12 days for

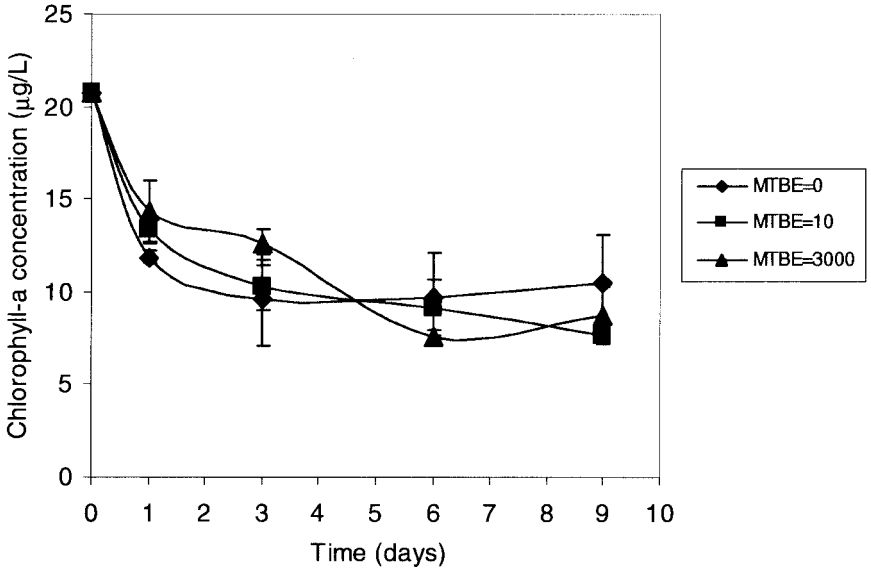

Figure 2. Average chlorophyll-*a* concentrations of Lake Texoma water collected from Soldier Creek marina as a function of MTBE concentrations in mg/L. Initial chlorophyll-*a* concentration was 20.8 µg/L.

Table 1. Activity rates for Catfish Bay algae and water in one-day

MTBE concentration (mg/L)	Activity rate, k (day^{-1})[a]
2000	- 0 -
4000	0.106
6000	0.535
8000	0.824
10000	0.150

[a] $k = \frac{2.3}{t}\log(\frac{C_0}{C_t})$, where t = time, C_o = initial concentration of chlorophyll-*a*, and C_t = concentration of chlorophyll-*a* at time t.

4000. The 2000 spike level did not reduce algae activity during the 6-day test period. Calculations of activity rate for one-day exposure were listed in Table 1.

It was demonstrated that MTBE would not be toxic to the lake water algae unless MTBE water concentrations were greater than 2000 mg/L. Typical concentrations of MTBE in the lake water used are 1 to 10 µg/L which will not have an apparent adverse influence on the algae biomass activity. In addition, the reduction of biomass chlorophyll-*a* in algae seems to be a good protocol to measure toxicity from chemical compounds.

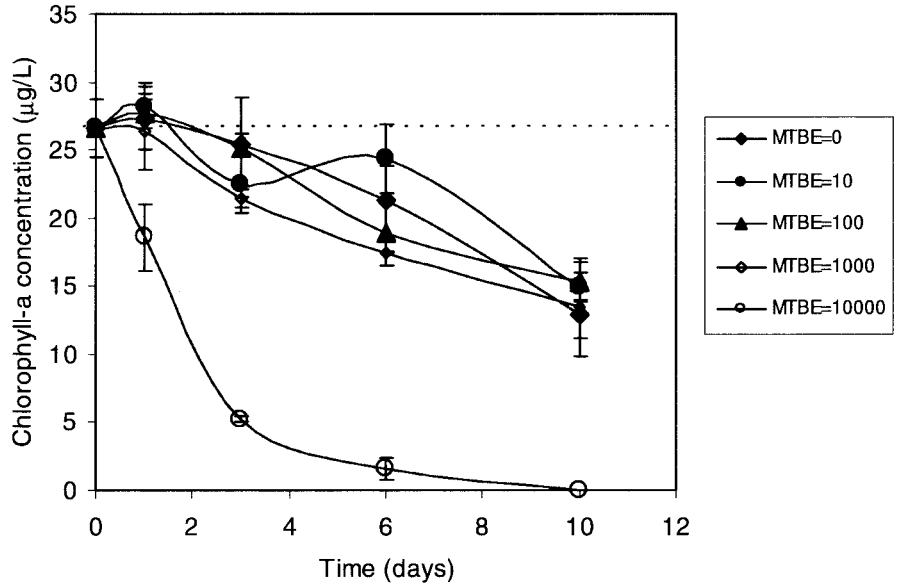

Figure 3. Average chlorophyll-*a* concentrations of Lake Texoma water collected from Cedar Mills marina as a function of MTBE concentrations in mg/L. Initial chlorophyll-*a* concentration was 26.6 µg/L.

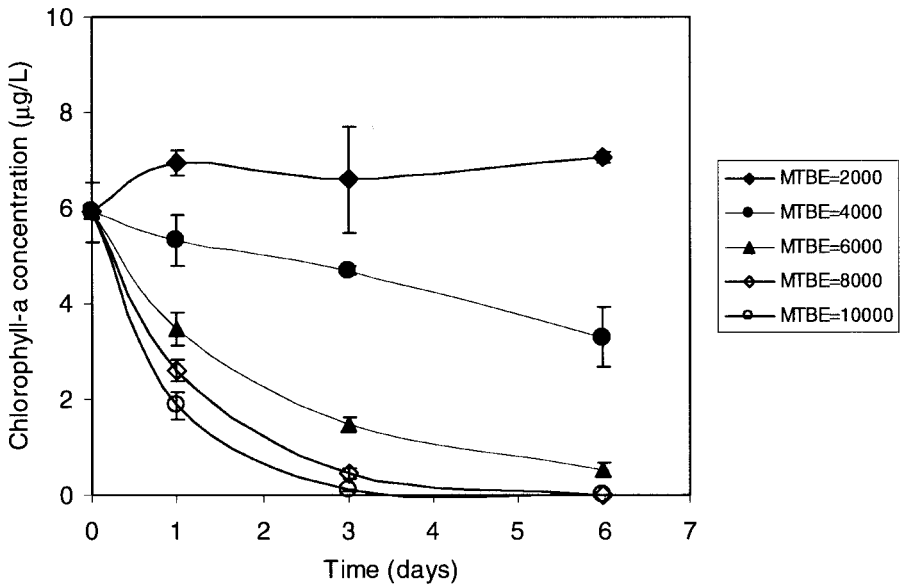

Figure 4. Average chlorophyll-*a* concentrations of Lake Texoma water collected from Catfish Bay marina as a function of MTBE concentrations in mg/L. Initial chlorophyll-*a* concentration was 5.91 µg/L.

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